A1
cont.

function was coupled to the amino modifier C6 phosphoramidite 13 (Figure 4d) using tetrazole as catalyst and subsequently oxidized with iodine water. The oligomer was cleaved from the support, and all the base-labile protecting groups were removed at the same time, by treating with conc. ammonia at 50°C. The terminal Mmt protecting group was then removed by treating with 80% acetic acid. 130 OD of the crude product was obtained, with this group product being purified by gel electrophoresis. 22.5 OD of product, having a molecular weight of 3303.8 (calc. 3305.0), was obtained.

A2

[090] The preparation was effected, in a 0.5 μmol synthesis, in an analogous manner to that described in Example 2. However, after synthesizing the carboxy terminus and the PNA moiety, a hydroxyethylglycine-based building block having thymine as the nucleobase (oegT) was coupled on in the last cycle. After eliminating the Dmt group, the free hydroxyl function was coupled to the biotin phosphoramidite 5 (Figure 4b) using tetrazole as catalyst and subsequently oxidized with iodine water and detritylated with trichloroacetic acid. The oligomer was cleaved from the support, and all the protecting groups were removed at the same time, by treating with conc. ammonia at 50°C. 37 OD of the crude product was obtained, with this crude product being purified by gel electrophoresis. 22.5 OD was obtained.

A3

[092] The synthesis was effected in analogy with Example 2 proceeding from the fluorescein-support 3 (Figure 6a and 8). The Dmt protecting group was eliminated from the fluorescein-support 3 by treating with 3% trichloroacetic acid. The free hydroxyl function was then reacted with the amino modifier C6 phosphoramidite 13 (4d) using tetrazole as catalyst.

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After condensation had taken place, oxidation was effected using an iodine solution (0.05 M in tetrahydrofuran/water, pyridine (7:2:1; v:v:v)). After that, the PNA moiety was prepared by solid phase synthesis as described in Example 1. A hydroxyethylglycine-based building block having thymine as nucleobase ((t)oeg) was coupled on in the last cycle. After eliminating the Dmt group, the free hydroxyl function was coupled to the phosphorylating reagent 1 (Figure 4a) using tetrazole as catalyst and subsequently oxidized with iodine water. Finally, the PNA was cleaved from the support, and the protecting groups were removed at the same time, by treating with conc. ammonia at 50°C overnight. 61 OD (260) of the crude product was obtained, with this crude product being purified by preparative polyacrylamide (PAA) gel electrophoresis. The desired product band was eluted with 0.2M triethylammonium bicarbonate buffer and desalted through a Bond-Elut C18 column (1 g). 5.6 OD was obtained. The product was analyzed by negative ion mass spectroscopy, which showed the calculated mass (calc. 3709.5; found 3706.3).

A3 cont.

[094] The synthesis was effected in analogy with Example 6 starting from 1 μmol of fluorescein support 3 (Figures 6a and 8). A hydroxyethylglycine-based building block having thymine as the nucleobase ((t)oeg) was coupled on in the last cycle. However, after eliminating the Dmt group, the free hydroxyl function was coupled to the C16 phosphorylating reagent 7 (Figure 4c) using tetrazole as catalyst and subsequently oxidized with iodine water. Finally, the PNA was eliminated from the support, and the protecting groups were removed at the same time, by treating with conc. ammonia at 50°C overnight. 61 OD (260) of the desired crude product was obtained, with this crude product being purified by preparative polyacrylamide (PAA) gel electrophoresis. The desired product band was eluted with 0.2M triethylammonium bicarbonate

A4

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